

Significance of DNA Adduct Studies in Animal Models for Cancer Molecular Dosimetry and Risk Assessment

by Frederick A. Beland¹ and Miriam C. Poirier²

To elucidate the relationship between DNA adduct formation and tumorigenesis, a number of experiments have been conducted to measure DNA adducts in target tissues from experimental animals during continuous exposure to carcinogens. With aflatoxins, aromatic amines, and polycyclic aromatic hydrocarbons, tumor induction appears to be associated with the major DNA adduct detected, whereas with *N*-nitrosamines the response is normally correlated with minor forms of DNA damage. During continuous carcinogen administration, steady-state adduct concentrations are generally obtained in the target tissues, and there is often a linear correlation between the carcinogen concentration and the steady-state DNA adduct level. Exceptions exist when the mechanism of activation changes or with the onset of significant toxicity. Steady-state DNA adduct levels are often linearly related to the tumorigenic response. Carcinogen-induced cell proliferation occurs when significant deviations from linearity are observed. Because DNA adducts detected in humans are chemically identical to those found in experimental animals, DNA adduct data in animals may contribute to our understanding of human cancer risk.

Introduction

Recently published reports have described the presence of DNA adducts in individuals exposed to chemical carcinogens [reviewed in Poirier and Weston (1)]. The majority of these carcinogen exposures appear to be chronic in nature, and the DNA adduct concentrations presumably reflect steady-state levels that are the product of concomitant adduct formation and removal. In spite of this achievement, the correlation between the measured DNA adduct levels and human cancer risk is not known and may be exceedingly complex. To help elucidate this relationship, a number of investigations have been conducted using animal models in which DNA adducts were measured during the chronic administration of tumorigenic dosing regimens. In this review, we discuss these studies, emphasizing four classes of chemical carcinogens for which there is substantial evidence of human exposure: *N*-nitrosamines, aflatoxins, aromatic amines, and polycyclic aromatic hydrocarbons. In addition, we will compare the DNA adducts that have been detected during these tumorigenic dosing studies with those identified in humans.

N-Nitrosamines

Humans are exposed to *N*-nitrosamines from a wide variety of sources including foods, beverages, tobacco, cosmetics, cutting oils, hydraulic fluids, and rubber products (2). As with the

majority of chemical carcinogens, *N*-nitrosamines tend to be chemically inert compounds that must be metabolized to reactive electrophiles before binding to cellular macromolecules (3). Although N7 guanine adducts or phosphotriesters typically are formed to the greatest extent, a comparison of the distribution of adducts from direct-acting alkylating agents with their tumorigenic potential indicates that alkylation of exocyclic oxygens (e.g., O⁶ of guanine) is more important for the induction of tumors than reaction with the ring nitrogens [e.g., N7 of guanine (4,5)].

The relationship between dose and the concentration of DNA adducts during continuous carcinogen administration has been investigated by Boucheron et al. (6), who treated rats with various concentrations of *N*-nitrosodiethylamine for up to 70 days. In the target organ, the liver, the concentration of O⁴-ethylthymidine increased rapidly at each dose for about the first 14 days, whereupon steady-state adduct levels were obtained (Fig. 1A). At steady state (e.g., after 49 days of continuous treatment; Fig. 1B), the levels of O⁴-ethylthymidine were dependent on dose, with essentially a linear correlation being observed between dose and adduct concentration up to 40 ppm. At 100 ppm *N*-nitrosodiethylamine, the steady-state levels of O⁴-ethylthymidine were similar to those at 40 ppm (Fig. 1B). This apparent failure to form more adducts may be due to increased cell turnover (7).

A similar study was conducted by Belinsky et al. (8), who measured O⁶-methylguanine in DNA from different cell types in the lungs of rats treated subcutaneously three times per week for 4 weeks with five different doses of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The concentration of

¹National Center for Toxicological Research, Jefferson, AR 72079.

²National Cancer Institute, Bethesda, MD 20892.

Address reprint requests to F. A. Beland, HFT-110, National Center for Toxicological Research, Jefferson, AR 72079.

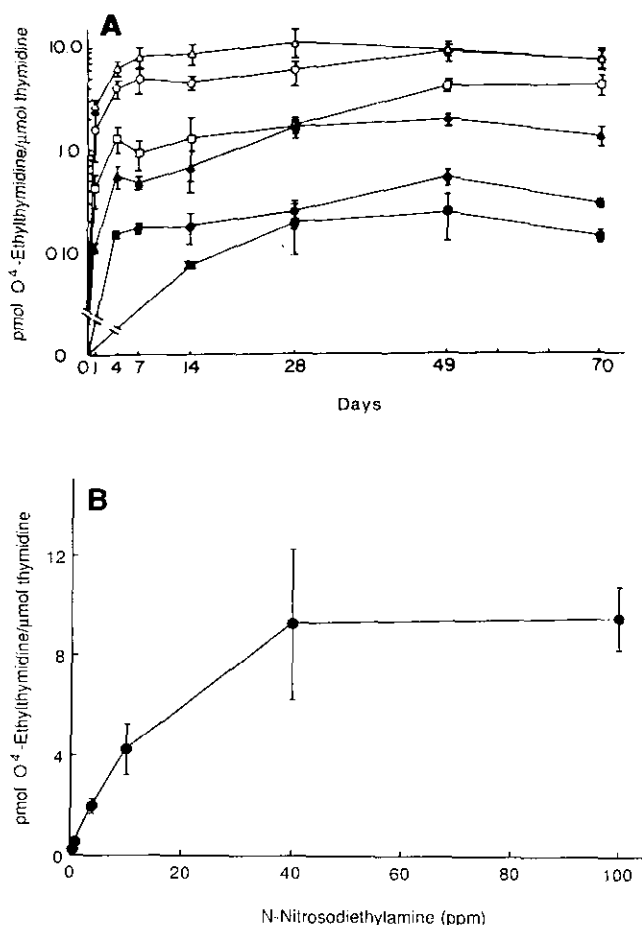


FIGURE 1. Accumulation of O^4 -ethylthymidine in hepatic DNA during continuous exposure of rats as a function of (A) time during exposure to 0.4 (■), 1 (●), 4 (▲), 10 (□), 40 (○), or 100 (△) ppm *N*-nitrosodiethylamine for up to 70 days or (B) dose after exposure from 0.4 to 100 ppm *N*-nitrosodiethylamine for 49 days. Data are from Boucheron et al. (6).

O^6 -methylguanine in Clara cells exhibited nonlinear kinetics with respect to dose, with adduct formation occurring to a much greater extent at lower doses than would have been expected from extrapolation of higher doses (Fig. 2A). This nonlinearity in response was attributed to the existence of a high-affinity pathway for the activation of NNK at low concentrations of the carcinogen. Since the kinetics of tumor induction (Fig. 2B) were similar to those of the adduct concentration in Clara cells (Fig. 2A), there was a linear correlation between the level of O^6 -methylguanine in these cells and the tumor incidence (Fig. 2C). In the liver and nasal passages, where NNK caused tumors only at high doses, adduct and tumor profiles did not correspond. This suggests that in these tissues both adduct formation and cytotoxicity are necessary for tumorigenesis.

In another study, in which rats were treated with four IP doses of NNK, a linear relationship was observed between hepatic concentrations of N^7 -methylguanine and amount of NNK given (9). This was not the situation in the lung, which, as in the Belinsky et al. study (8), was attributed to the existence of a high-affinity activation pathway in lung tissue at low doses of NNK.

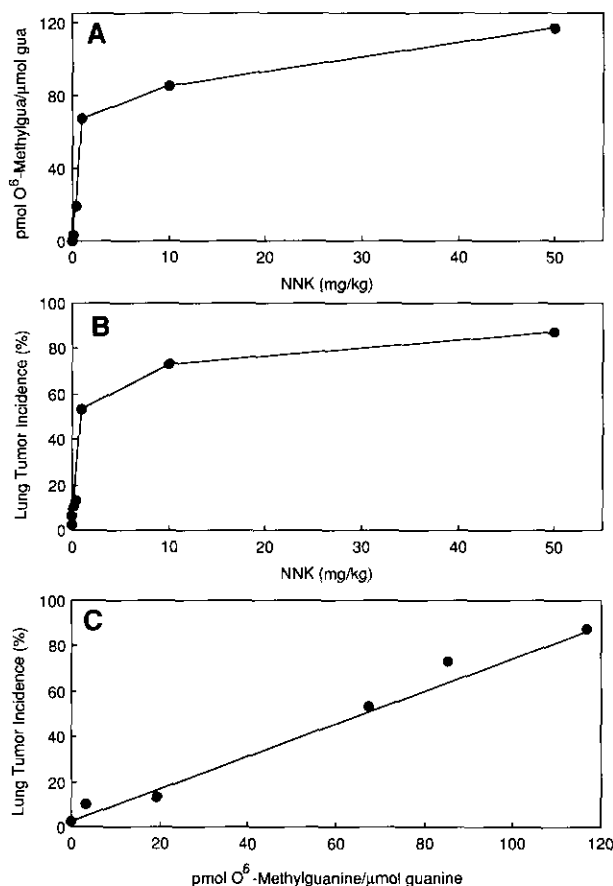


FIGURE 2. Relationship between (A) administered dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and concentration of O^6 -methylguanine in Clara cells of rats after dosing three times per week for 4 weeks; (B) administered dose of NNK and lung tumor incidence after 2 years following dosing three times per week for 20 weeks; and (C) concentration of O^6 -methylguanine in Clara cells of rats after dosing three times per week for 4 weeks and lung tumor incidence after 2 years following dosing three times per week for 20 weeks. Data are from Belinsky et al. (8).

With the exception of the induction of oral cancer in snuff dip-pers (10,11), there is no conclusive epidemiological evidence for the carcinogenicity of *N*-nitrosamines in humans. Nevertheless, the types of DNA adducts formed in exposed individuals are similar to those observed in experimental animals. For example, Herron and Shank (12) found N^7 - and O^6 -methylguanine in the liver of a victim poisoned with *N*-nitrosodimethylamine. More recently, Umberhauer et al. (13) detected O^6 -methylguanine in stomach and esophageal tissue and esophageal tumor DNA from Chinese cancer patients. This adduct was also found at lower levels in the same tissues in Europeans, who are at a lower risk for developing esophageal tumors. Similar results were obtained by Saffhill et al. (14) when comparing individuals in Southeast Asia with those from England. Likewise, Huh et al. (15) found higher levels of O^4 -ethylthymine in DNA from Japanese liver cancer patients compared to nontumor-bearing control patients. O^6 -Methylguanine and O^6 -ethylguanine have also been detected in peripheral lung DNA of smokers and nonsmokers, but the concentrations were not dependent on an individual's smoking status (16).

Aflatoxins

Humans are exposed to aflatoxins through the consumption of moldy cereals, grains, and nuts (17,18). Four major naturally occurring aflatoxins, aflatoxin B₁ (AFB₁), aflatoxin B₂, aflatoxin G₁, and aflatoxin G₂, have been characterized, with AFB₁ being the most abundant as well as the most carcinogenic. The metabolic activation of AFB₁ involves oxidation of the 8,9-olefinic bond to give AFB₁-8,9-oxide (19), which reacts with DNA to yield *trans*-8,9-dihydro-8-(deoxyguanosin-7-yl)-9-hydroxy AFB₁ [AFB₁-N7-dG (20)]. AFB₁-N7-dG carries a positive charge and is therefore unstable. It can undergo depurination to give an apurinic site within the DNA (20) or base-catalyzed opening of the imidazole ring to yield pyrimidine adducts (21).

In experimental animals, AFB₁ is normally hepatocarcinogenic with the relative order of sensitivity being: trout > rat >> hamster \approx mouse \approx salmon (17,22). During the continuous administration of AFB₁, steady-state hepatic DNA adduct levels are obtained in approximately 2–6 weeks in rats (23,24) and 3 weeks in trout (22). In both species the steady-state adduct concentrations appear to be linearly related to the concentration of AFB₁ administered chronically (24–26). Moreover, if the steady-state adduct levels are compared to the hepatic tumor incidence, a nearly identical linear relationship is obtained for both species (27).

In humans, there is a positive correlation between the amount of AFB₁ ingested and the incidence of liver cancer (17,18). Furthermore, AFB₁ DNA adducts have been detected in tissues and urine from exposed humans (28–30). More recently, a highly significant correlation was observed between daily consumption of AFB₁ and concentration of serum adducts of AFB₁ and urinary AFB₁-N7-Gua (31,32), which presumably represent steady-state levels.

Aromatic Amines

Human exposure to aromatic amines and amides occurs from a number of sources including various industrial processes, cigarette smoke, and certain foods (33). There is also widespread exposure to nitropolycyclic aromatic hydrocarbons, which are products of incomplete combustion and are converted to aromatic amines by nitroreduction (33). In experimental animals, major target organs for tumor induction from these compounds include the liver, urinary bladder, mammary gland, and intestine. In these tissues, the major adducts are formed through covalent linkage of the amine or amide nitrogen to C-8 of guanine, while minor adducts arise from reactions between the carbons *ortho* to the amine or amide nitrogen and the exocyclic nitrogens and oxygens of guanine and adenine (33).

During the continuous administration of aromatic amine carcinogens to rodents, steady-state blood (34,35), tissue (34), hepatic DNA adduct (36–38), and bladder DNA adduct (38) concentrations are obtained after approximately one month of dosing. These steady-state levels are dose-related (34,37,38) (Fig. 3A), and a linear correlation exists between the DNA adduct concentration and the hepatic tumor incidence in rats (37) and female mice (38) (Fig. 3B) administered 2-acetylaminofluorene and in female mice (39) treated with 4-aminobiphenyl. However, in female mice fed 2-acetylaminofluorene (38) (Fig.

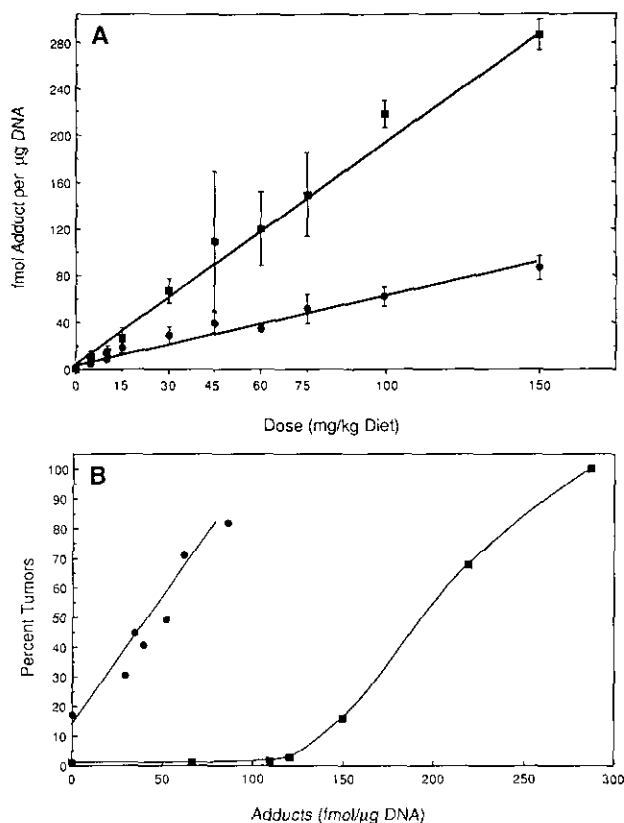


FIGURE 3. Relationship between (A) the dose of 2-acetylaminofluorene fed to female mice for 28 days and the concentration of *N*-(deoxyguanosin-8-yl)-2-aminofluorene in liver (●) and bladder (■) DNA and (B) concentration of *N*-(deoxyguanosine-8-yl)-2-aminofluorene in liver (●) and bladder (■) of female mice fed 2-acetylaminofluorene for 28 days and the tumor incidence in the same tissues after feeding the same concentrations for 33 months. Data are from Poirier et al. (38).

3B) and male mice administered 4-aminobiphenyl (39), the relationship between the bladder tumor yield and DNA adduct concentration is nonlinear. This nonlinearity in response appears to be associated with the induction of hyperplasia and implicates cell proliferation as an additional factor in the induction of bladder cancer in mice.

Aromatic amines are clearly associated with the induction of urinary bladder cancer in humans (40). Hemoglobin adducts of 4-aminobiphenyl have been detected in blood samples from humans, and these concentrations are higher in smokers as compared to nonsmokers (41–43), which is consistent with tobacco-related increases in the incidence of bladder cancer (44). In other studies, ³²P-postlabeling and immunoassays have indicated the presence of 4-aminobiphenyl DNA adducts in human lung and bladder samples (16,45,46), with the levels in the bladder being significantly higher in smokers (46).

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are byproducts of combustion processes resulting in ubiquitous human exposure to this class of carcinogens (47). In experimental animals, PAHs induce primarily skin, stomach, lung, and mammary gland

tumors (48), and this appears to be the result of metabolic conversion to dihydrodiol epoxides (49). Generally, guanine is the preferred base for adduct formation with these reactive electrophiles; however, depending on the PAH, considerable binding can also occur with adenine and cytosine. Furthermore, because dihydrodiol epoxides are optically active (49), the extent of reaction with a particular nucleic acid base will depend on the particular stereoisomer being considered (50).

Recently, the kinetics of DNA adduct formation have been examined in the epidermis of mice administered benzo[a]pyrene topically once a week for 29 weeks (51). Steady-state adduct levels appeared to be obtained after 3 weeks of dosing, and the adduct levels were linearly related to dose up to 32 μg per treatment, whereupon a plateau occurred. As the skin tumor incidence was not linearly correlated to the dose, it was suggested that cell proliferation plays an essential role in the induction of these tumors. In preliminary studies in our laboratory, a linear relationship has been found between the adduct levels in lung and forestomach and the concentration of benzo[a]pyrene fed to mice for 1 month at doses up to 50 mg/kg diet (Culp and Beland, unpublished observation). The induction of forestomach tumors in mice appears to be markedly nonlinear with dose (52); thus, as with bladder tumors in mice (38,39), cell proliferation may be an additional critical factor for tumorigenesis in this tissue.

PAHs are probably carcinogenic in humans (53), and DNA adducts from these compounds have been detected in human populations (1). Inasmuch as cigarette smoke contains substantial quantities of PAHs (54), a number of studies have compared PAH-DNA adduct concentrations in various tissues from smokers and nonsmokers. Immunoassays with antibodies elicited against (\pm)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide-modified DNA have revealed that smokers often have somewhat higher PAH-DNA adduct levels than nonsmokers (55-59), although this is not always the case (16,60-64). More dramatic differences have been found using ^{32}P -postlabeling, which is generally specific for "aromatic" DNA adducts. In several studies, smokers have had higher levels of discrete adducts (55,56,65) or a larger diffuse area of adducts (58,66-68) than nonsmokers; however, in only one of these studies (58) was a benzo[a]pyrene dihydrodiol epoxide-derived adduct detected. The presence of benzo[a]pyrene dihydrodiol epoxide DNA adducts in human placenta (69) and lung (70) has been confirmed by subjecting the DNA to immunoaffinity chromatography followed by high-pressure liquid chromatography and synchronous fluorescence spectroscopy, and also by gas chromatography-mass spectrometry in the case of the placental DNA; however, the extent of smoking did not always correlate with the adduct concentrations observed.

In another study (71), benzo[a]pyrene diol epoxide adducts were detected in alveolar macrophages by synchronous fluorescence spectrometry, and the levels did appear to depend upon smoking status. Other exposure-related increases in PAH-DNA adducts, as determined by immunoassays, have been reported in peripheral blood lymphocytes from iron foundry workers and individuals ingesting charcoal-broiled beef, and positive immunochemical results have also been found with blood samples from coke-oven workers, foundry workers, roofers, and firefighters (1).

Summary

In this review we have considered the DNA adducts formed in animal models from four classes of chemical carcinogens for which there is substantial evidence for human exposure. Some interesting similarities and differences between carcinogen-DNA interactions for these classes of compounds are discussed below.

The site of substitution for biologically important adducts appears to be chemical-class specific. Thus, tumor induction from *N*-nitrosamines is best associated with *O*⁶-guanine and *O*⁴-thymine substitution. For aflatoxins, the response is correlated with reaction at *N*7 of guanine; with aromatic amines, *C*8-guanine substitution generally appears to be the critical lesion; and for PAHs, *N*2 of guanine and/or *N*6 of adenine appear to be the important sites for substitution.

Tumors induced by *N*-nitrosamines are associated with the formation of minor adducts (e.g., *O*⁶-alkylguanine and *O*⁴-alkylthymine), whereas with the other classes of carcinogens, tumor induction is normally associated with the major forms of DNA damage.

Steady-state DNA adduct concentrations are obtained during continuous carcinogen administration, and this typically occurs after approximately 1 month of chronic dosing. Because DNA adduct levels reach steady state, an estimation of the risk for developing a tumor cannot be obtained from the DNA adduct concentration by itself, but has to include both the DNA adduct concentration and the length of carcinogen exposure.

There is often a linear correlation between the chronically administered dose levels and the steady-state DNA adduct concentrations in target tissues (e.g., 2-acetylaminofluorene in mouse liver and bladder; Fig. 3A). Exceptions exist when the mechanism of activation changes (e.g., NNK in rat lung; Fig. 2A) or with the onset of significant toxicity (e.g., *N*-nitrosodiethylamine in rat liver; Fig. 1B).

Steady-state DNA adduct levels are often linearly related to the tumorigenic response (e.g., 2-acetylaminofluorene in mouse liver; Fig. 3B). When significant deviations from linearity are observed (e.g., 2-acetylaminofluorene in mouse bladder; Fig. 3B), carcinogen-induced cell proliferation appears to be an additional component for tumorigenesis. Because this response occurs at relatively high carcinogen doses, it may not be germane to most human exposure scenarios.

DNA adducts can be detected in humans, and these adducts correspond to those found in experimental animals. It should be possible, therefore, to use DNA adduct data in animals to estimate human cancer risk.

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October-1 November 1991.

We thank Cindy Hartwick and Pat Fleischer for helping to prepare this review.

REFERENCES

1. Poirier, M. C., and Weston, A. DNA adduct determination in humans. *Prog. Clin. Biol. Res.* 374: 205-218 (1991).
2. Preussmann, R., and Eisenbrand, G. *N*-Nitroso carcinogens in the environment. In: *Chemical Carcinogens*, Vol. 2, ACS Monograph 182 (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1984, pp. 829-868.
3. Lawley, P. D. *N*-Nitroso compounds. In: *Handbook of Experimental Phar-*

- macology, Vol. 94/1: Chemical Carcinogenesis and Mutagenesis I (C. S. Cooper and P. L. Grover, Eds.), Springer-Verlag, Heidelberg, 1990, pp. 409-469.
4. Frei, J. V., and Lawley, P. D. Tissue distribution and mode of DNA methylation in mice by methyl methanesulphonate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine: lack of thymic lymphoma induction and low extent of methylation of target tissue DNA at O-6 of guanine. *Chem.-Biol. Interact.* 13: 215-222 (1976).
5. Frei, J. V., Swenson, D. H., Warren, W., and Lawley, P. D. Alkylation of deoxyribonucleic acid *in vivo* in various organs of C57BL mice by the carcinogens *N*-methyl-*N*-nitrosourea, *N*-ethyl-*N*-nitrosourea and ethyl methanesulphonate in relation to induction of thymic lymphoma. Some applications of high-pressure liquid chromatography. *Biochem. J.* 174: 1031-1044 (1978).
6. Boucheron, J. A., Richardson, F. C., Morgan, P. H., and Swenberg, J. A. Molecular dosimetry of *O*⁶-ethyldeoxythymidine in rats continuously exposed to diethylnitrosamine. *Cancer Res.* 47: 1577-1581 (1987).
7. Deal, F. H., Richardson, F. C., and Swenberg, J. A. Dose response of hepatocyte replication in rats following continuous exposure to diethylnitrosamine. *Cancer Res.* 49: 6985-6988 (1989).
8. Belinsky, S. A., Foley, J. F., White, C. M., Anderson, M. W., and Maronpot, R. R. Dose-response relationship between *O*⁶-methylguanine formation in Clara cells and induction of pulmonary neoplasia in the rat by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.* 50: 3772-3780 (1990).
9. Murphy, S. E., Palomino, A., Hecht, S. S., and Hoffmann, D. Dose-response study of DNA and hemoglobin adduct formation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in F344 rats. *Cancer Res.* 50: 5446-5452 (1990).
10. Winn, D. M. Smokeless tobacco and oral/pharynx cancer: the role of cofactors. In: *Mechanisms in Tobacco Carcinogenesis*, Banbury Report 23 (D. Hoffmann and C. C. Harris, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986, pp. 361-375.
11. Montesano, R., Br sil, H., Degan, P., Martel-Planche, G., Serres, M., and Wild, C. P. Detection in human cells of alkylated macromolecules attributable to exposure to nitrosamines. In: *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, IARC Scientific Publication No. 89 (H. Bartsch, K. Hemminki, and I. K. O'Neill, Eds.), International Agency for Research on Cancer, Lyon, 1988, pp. 75-82.
12. Herron, D. C., and Shank, R. C. Methylated purines in human liver DNA after probable dimethylnitrosamine poisoning. *Cancer Res.* 40: 3116-3117 (1980).
13. Umbenhauer, D., Wild, C. P., Montesano, R., Saffhill, R., Boyle, J. M., Huh, N., Kirshtein, U., Thomale, J., Rajewsky, M. F., and Lu, S. H. *O*⁶-Methyldeoxyguanosine in oesophageal DNA among individuals at high risk of oesophageal cancer. *Int. J. Cancer* 36: 661-665 (1985).
14. Saffhill, R., Badawi, A. F., and Hall, C. N. Detection of *O*⁶-methylguanine in human DNA. In: *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, IARC Scientific Publications No. 89 (H. Bartsch, K. Hemminki, and I. K. O'Neill, Eds.), International Agency for Research on Cancer, Lyon, 1988, pp. 301-305.
15. Huh, N., Satoh, M. S., Shiga, J., and Kuroki, T. Detection of *O*⁶-ethylthymine in human liver DNA. In: *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, IARC Scientific Publications No. 89 (H. Bartsch, K. Hemminki, and I. K. O'Neill, Eds.), International Agency for Research on Cancer, Lyon, 1988, pp. 292-295.
16. Wilson, V. L., Weston, A., Manchester, D. K., Trivers, G. E., Roberts, D. W., Kadlubar, F. F., Wild, C. P., Montesano, R., Willey, J. C., Mann, D. L., and Harris, C. C. Alkyl and aryl carcinogen adducts detected in human peripheral lung. *Carcinogenesis* 10: 2149-2153 (1989).
17. Busby, W. F., Jr., and Wogan, G. N. Aflatoxins. In: *Chemical Carcinogens*, ACS Monograph 182 (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1984, pp. 945-1136.
18. Groopman, J. D., and Cain, L. G. Interactions of fungal and plant toxins with DNA: aflatoxins, sterigmatocystin, safrole, cycasin, and pyrrolizidine alkaloids. In: *Handbook of Experimental Pharmacology*, Vol. 94/1: Chemical Carcinogenesis and Mutagenesis I (C. S. Cooper and P. L. Grover, Eds.), Springer-Verlag, Heidelberg, 1990, pp. 373-407.
19. Baertschi, S. W., Raney, K. D., Stone, M. P., and Harris, T. M. Preparation of the 8,9-epoxide of the mycotoxin aflatoxin B₁: the ultimate carcinogenic species. *J. Am. Chem. Soc.* 110: 7929-7931 (1988).
20. Essigmann, J. M., Croy, R. G., Nadzan, A. M., Busby, W. F., Jr., Reinhold, V. N., B chi, G., and Wogan, G. N. Structural identification of the major DNA adduct formed by aflatoxin B₁ *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* 74: 1870-1874 (1977).
21. Hertzog, P. J., Smith, J. R. L., and Garner, R. C. Characterisation of the imidazole ring-opened forms of *trans*-8,9-dihydro-8-(7-guanyl)9-hydroxy aflatoxin B₁. *Carcinogenesis* 3: 723-725 (1982).
22. Bailey, G. S., Williams, D. E., Wilcox, J. S., Loveland, P. M., Coulombe, R. A., and Hendricks, J. D. Aflatoxin B₁ carcinogenesis and its relation to DNA adduct formation and adduct persistence in sensitive and resistant salmonid fish. *Carcinogenesis* 9: 1919-1926 (1988).
23. Wild, C. P., Garner, R. C., Montesano, R., and Tursi, F. Aflatoxin B₁ binding to plasma albumin and liver DNA upon chronic administration to rats. *Carcinogenesis* 7: 853-858 (1986).
24. Buss, P., Caviezel, M., and Lutz, W. K. Linear dose-response relationship for DNA adducts in rat liver from chronic exposure to aflatoxin B₁. *Carcinogenesis* 11: 2133-2135 (1990).
25. Dashwood, R. H., Arbogast, D. N., Fong, A. T., Hendricks, J. D., and Bailey, G. S. Mechanisms of anti-carcinogenesis by indole-3-carbinol: detailed *in vivo* DNA binding dose-response studies after dietary administration with aflatoxin B₁. *Carcinogenesis* 9: 427-432 (1988).
26. Dashwood, R. H., Arbogast, D. N., Fong, A. T., Pereira, C., Hendricks, J. D., and Bailey, G. S. Quantitative inter-relationships between aflatoxin B₁ carcinogen dose, indole-3-carbinol anti-carcinogen dose, target organ DNA adduction and final tumor response. *Carcinogenesis* 10: 175-181 (1989).
27. Bechtel, D. H. Molecular dosimetry of hepatic aflatoxin B₁-DNA adducts: linear correlations with hepatic cancer risk. *Regul. Toxicol. Pharmacol.* 10: 74-81 (1989).
28. Aulup, H., Bradley, K. A., Shamsuddin, A. K. M., Wakhisi, J., and Wasumna, A. Detection of putative adduct with fluorescence characteristics identical to 2,3-dihydro-2-(7'-guanyl)-3-hydroxyaflatoxin B₁ in human urine collected in Murang'a district, Kenya. *Carcinogenesis* 4: 1193-1195 (1983).
29. Groopman, J. D., Donahue, P. R., Zhu, J., Chen, J., and Wogan, G. N. Aflatoxin metabolism in humans: detection of metabolites and nucleic acid adducts in urine by affinity chromatography. *Proc. Natl. Acad. Sci. U.S.A.* 82: 6492-6496 (1985).
30. Hsieh, L.-L., Hsu, S.-W., Chen, D.-S., and Santella, R. M. Immunological detection of aflatoxin B₁-DNA adducts formed *in vivo*. *Cancer Res.* 48: 6328-6331 (1988).
31. Gan, L.-S., Skipper, P. L., Peng, X., Groopman, J. D., Chen, J.-S., Wogan, G. N., and Tannenbaum, S. R. Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B₁ intake and urinary excretion of aflatoxin M₁. *Carcinogenesis* 9: 1323-1325 (1988).
32. Groopman, J. D., Sabbioni, G., and Wild, C. P. Molecular dosimetry of human aflatoxin exposures. In: *Molecular Dosimetry and Human Cancer: Analytical, Epidemiological, and Social Considerations* (J. D. Groopman and P. L. Skipper, Eds.), CRC Press, Boca Raton, FL, 1990, pp. 303-324.
33. Beland, F. A., and Kadlubar, F. F. Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons. In: *Handbook of Experimental Pharmacology*, Vol. 94/1: Chemical Carcinogenesis and Mutagenesis I (C. S. Cooper and P. L. Grover, Eds.), Springer-Verlag, Heidelberg, 1990, pp. 267-325.
34. Jackson, C. D., Weis, C., and Shellenberger, T. E. Tissue binding of 2-acetylaminofluorene in BALB/c and C57B1/6 mice during chronic oral administration. *Chem.-Biol. Interact.* 32: 63-81 (1980).
35. Green, L. C., Skipper, P. L., Turesky, R. J., Bryant, M. S., and Tannenbaum, S. R. *In vivo* dosimetry of 4-aminobiphenyl in rats via a cysteine adduct in hemoglobin. *Cancer Res.* 44: 4254-4259 (1984).
36. Poirier, M. C., Hunt, J. M., True, B., Laishes, B. A., Young, J. F., and Beland, F. A. DNA adduct formation, removal and persistence in rat liver during one month of feeding 2-acetylaminofluorene. *Carcinogenesis* 5: 1591-1596 (1984).
37. Buss, P., and Lutz, W. K. Steady-state DNA adduct level in rat liver after chronic exposure to low doses of aflatoxin B₁ and 2-acetylaminofluorene. *Proc. Am. Assoc. Cancer Res.* 29: 96 (1988).
38. Poirier, M. C., Fullerton, N. F., Kinouchi, T., Smith, B. A., and Beland, F. A. Comparison between DNA adduct formation and tumorigenesis in livers and bladders of mice chronically fed 2-acetylaminofluorene. *Carcinogenesis* 12: 895-900 (1991).
39. Beland, F. A., Fullerton, N. F., Smith, B. A., and Poirier, M. C. DNA adduct formation and aromatic amine tumorigenesis. In: *Relevance of Animal Studies*

- to the Evaluation of Human Cancer Risk (R. D'Amato, T. J. Slaga, W. Farland, and C. Henry, Eds.), John Wiley and Sons, New York, 1992, pp. 79-92.
40. Parkes, H. G., and Evans, A. E. J. Epidemiology of aromatic amine cancers. In: *Chemical Carcinogens*, ACS Monograph 182 (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1984, pp. 277-301.
 41. Bryant, M. S., Skipper, P. L., Tannenbaum, S. R., and Maclure, M. Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res.* 47: 602-608 (1987).
 42. Bryant, M. S., Vineis, P., Skipper, P. L., and Tannenbaum, S. R. Hemoglobin adducts of aromatic amines: associations with smoking status and type of tobacco. *Proc. Natl. Acad. Sci. U.S.A.* 85: 9788-9791 (1988).
 43. Vineis, P., Caporaso, N., Tannenbaum, S. R., Skipper, P. L., Glogowski, J., Bartsch, H., Coda, M., Talaska, G., and Kadlubar, F. Acetylation phenotype, carcinogen-hemoglobin adducts, and cigarette smoking. *Cancer Res.* 50: 3002-3004 (1990).
 44. Mommsen, S., and Aagaard, J. Tobacco as a risk factor in bladder cancer. *Carcinogenesis* 4: 335-383 (1983).
 45. Kadlubar, F. F., Talaska, G., Lang, N. P., Benson, R. W., and Roberts, D. W. Assessment of exposure and susceptibility to aromatic amine carcinogens. In: *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, IARC Scientific Publication No. 89 (H. Bartsch, K. Hemminki, and I. K. O'Neill, Eds.), International Agency for Research on Cancer, Lyon, 1988, pp. 166-174.
 46. Talaska, G., Al-Juburi, A. Z. S., and Kadlubar, F. F. Smoking related carcinogen-DNA adducts in biopsy samples of human urinary bladder: identification of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. *Proc. Natl. Acad. Sci. U.S.A.* 88: 5350-5354 (1991).
 47. Dipple, A. Polycyclic aromatic hydrocarbon carcinogenesis: an introduction. In: *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Symposium Series 283 (R. G. Harvey, Ed.), American Chemical Society, Washington, DC, 1985, pp. 1-17.
 48. Dipple, A., Moschel, R. C., and Bigger, C. A. H. Polynuclear aromatic carcinogens. In: *Chemical Carcinogens*, ACS Monograph 182 (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1984, pp. 41-163.
 49. Hall, M., and Grover, P. L. Polycyclic aromatic hydrocarbons: metabolism, activation and tumor initiation. In: *Handbook of Experimental Pharmacology*, Vol. 94/1: *Chemical Carcinogenesis and Mutagenesis I* (C. S. Cooper, and P. L. Grover, Eds.), Springer-Verlag, Heidelberg, 1990, pp. 327-372.
 50. Dipple, A., Pigott, M. A., Agarwal, S. K., Yagi, H., Sayer, J. M., and Jerina, D. M. Optically active benzo[*c*]phenanthrene diol epoxides bind extensively to adenine in DNA. *Nature* 327: 535-536 (1987).
 51. Albert, R. E., Miller, M. L., Cody, T., Andringa, A., Shukla, R., and Baxter, C. S. Benzo[*a*]pyrene-induced skin damage and tumor promotion in the mouse. *Carcinogenesis* 12: 1273-1280 (1991).
 52. Neal, J., and Rigdon, R. H. Gastric tumors in mice fed benzo[*a*]pyrene: a quantitative study. *Texas Rep. Biol. Med.* 25: 553-557 (1967).
 53. IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 32. *Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data*. International Agency for Research on Cancer, Lyon, 1983, pp. 1-477.
 54. U.S. Surgeon General. *The Health Consequences of Smoking. Cancer. A Report of the Surgeon General*, U.S. Department of Health and Human Services, Rockville, MD, 1982, pp. 193-233.
 55. Everson, R. B., Randerath, E., Santella, R. M., Cefalo, R. C., Avitts, T. A., and Randerath, K. Detection of smoking-related covalent DNA adducts in human placenta. *Science* 231: 54-57 (1986).
 56. Everson, R. B., Randerath, E., Santella, R. M., Avitts, T. A., Weinstein, I. B., and Randerath, K. Quantitative associations between DNA damage in human placenta and maternal smoking and birth weight. *J. Natl. Cancer Inst.* 80: 567-576 (1988).
 57. Perera, F. P., Santella, R. M., Brenner, D., Poirier, M. C., Munshi, A. A., Fischman, H. K., and Van Ryzin, J. V. DNA adducts, protein adducts, and sister chromatid exchange in cigarette smokers and nonsmokers. *J. Natl. Cancer Inst.* 79: 449-456 (1987).
 58. van Schooten, F. J., Hillebrand, M. J. X., van Leeuwen, F. E., Lutgerink, J. T., van Zandwijk, N., Jansen, H. M., and Kriek, E. Polycyclic aromatic hydrocarbon-DNA adducts in lung tissue from lung cancer patients. *Carcinogenesis* 11: 1677-1681 (1990).
 59. van Schooten, F. J., van Leeuwen, F. E., Hillebrand, M. J. X., de Rijke, M. E., Hart, A. A. M., van Veen, H. G., Oosterink, S., and Kriek, E. Determination of benzo[*a*]pyrene diol epoxide-DNA adducts in white blood cell DNA from coke-oven workers: the impact of smoking. *J. Natl. Cancer Inst.* 82: 927-933 (1990).
 60. Perera, F. P., Poirier, M. C., Yuspa, S. H., Nakayama, J., Jaretski, A., Curnen, M. M., Knowles, D. M., and Weinstein, I. B. A pilot project in molecular cancer epidemiology: determination of benzo[*a*]pyrene-DNA adducts in animal and human tissues by immunoassays. *Carcinogenesis* 3: 1405-1410 (1982).
 61. Perera, F. P., Hemminki, K., Young, T. L., Brenner, D., Kelly, G., and Santella, R. M. Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundry workers. *Cancer Res.* 48: 2288-2291 (1988).
 62. Harris, C. C., Vahakangas, K., Newnham, M. J., Trivers, G. E., Shamsuddin, A., Sinopoli, N., Mann, D. L., and Wright, W. E. Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. *Proc. Natl. Acad. Sci. U.S.A.* 82: 6672-6676 (1985).
 63. Shamsuddin, A. K. M., Sinopoli, N. T., Hemminki, K., Boesch, R. R., and Harris, C. C. Detection of benzo[*a*]pyrene: DNA adducts in human white blood cells. *Cancer Res.* 45: 66-68 (1985).
 64. Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G. E., Newman, M. J., and Harris, C. C. Determination of polycyclic aromatic hydrocarbons in the urine, benzo[*a*]pyrene diol epoxide-DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. *Cancer Res.* 46: 4178-4183 (1986).
 65. Randerath, E., Avitts, T. A., Reddy, M. V., Miller, R. H., Everson, R. B., and Randerath, K. Comparative ³²P-analysis of cigarette smoke-induced DNA damage in human tissues and mouse skin. *Cancer Res.* 46: 5869-5877 (1986).
 66. Phillips, D. H., Hewer, A., Martin, C. N., Garner, R. C., and King, M. M. Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature* 336: 790-792 (1988).
 67. Randerath, E., Miller, R. H., Mittal, D., Avitts, T. A., Dunsford, H. A., and Randerath, K. Covalent DNA damage in tissues of cigarette smokers as determined by ³²P-postlabeling assay. *J. Natl. Cancer Inst.* 81: 341-347 (1989).
 68. Dunn, B. P., Vedal, S., San, R. H. C., Kwan, W.-F., Nelems, B., Enarson, D. A., and Stich, H. F. DNA adducts in bronchial biopsies. *Int. J. Cancer* 48: 485-492 (1991).
 69. Manchester, D. K., Weston, A., Choi, J.-S., Trivers, G. E., Fennessey, P. V., Quintana, E., Farmer, P. B., Mann, D. L., and Harris, C. C. Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in human placenta. *Proc. Natl. Acad. Sci. U.S.A.* 85: 9243-9247 (1988).
 70. Weston, A., and Bowman, E. D. Fluorescence detection of benzo[*a*]pyrene-DNA adducts in human lung. *Carcinogenesis* 12: 1445-1449 (1991).
 71. Izzotti, A., Rossi, G. A., Bagnasco, M., and De Flora, S. Benzo[*a*]pyrene diol epoxide-DNA adducts in alveolar macrophages of smokers. *Carcinogenesis* 12: 1281-1285 (1991).